

WHAT IS CLAIMED IS:

1 1. A *Lactobacillus* bacterium comprising an expression cassette, the
2 expression cassette comprising a promoter operably linked to polynucleotide encoding a
3 signal sequence and a biologically-active polypeptide, wherein the biologically active
4 polypeptide is linked to a heterologous carboxyl terminal cell wall targeting region and
5 wherein the heterologous carboxyl terminal cell wall targeting region comprises in the
6 following order:

7 a cell wall associated sequence;
8 LPQ(S/A/T)(G/A);and
9 a hydrophobic sequence.

1 2. The *Lactobacillus* bacterium of claim 1, wherein the cell wall
2 associated sequence comprises at least 50 amino acids.

1 3. The *Lactobacillus* bacterium of claim 1, wherein the cell wall
2 associated sequence comprises at least 200 amino acids.

1 4. The *Lactobacillus* bacterium of claim 1, wherein the heterologous
2 carboxyl terminal cell wall targeting region further comprises a charged sequence at the
3 carboxyl terminus of region.

1 5. The *Lactobacillus* bacterium of claim 1, wherein the *Lactobacillus*
2 bacterium is a vagina-colonizing strain.

1 6. The *Lactobacillus* bacterium of claim 1, wherein the *Lactobacillus*
2 bacterium is selected from the group consisting of *L. jensenii*, *L. gasseri*, and *L. casei*.

1 7. The *Lactobacillus* bacterium of claim 1, wherein the cell wall targeting
2 region comprises the amino acid sequence LPQSG.

1 8. The *Lactobacillus* bacterium of claim 1, wherein the cell wall targeting
2 region comprises the amino acid sequence LPQAG.

1 9. The *Lactobacillus* bacterium of claim 1, wherein the cell wall targeting
2 region comprises the amino acid sequence LPQTG.

- 1 10. The *Lactobacillus* bacterium of claim 1, wherein the cell wall targeting
2 region comprises the amino acid sequence LPQTA.
- 1 11. The *Lactobacillus* bacterium of claim 1, wherein the cell wall targeting
2 region comprises SEQ ID NO:7.
- 1 12. The *Lactobacillus* bacterium of claim 1, wherein the cell wall targeting
2 region comprises SEQ ID NO:8.
- 1 13. The *Lactobacillus* bacterium of claim 1, wherein the biologically
2 active polypeptide is expressed in the cell wall of the bacterium.
- 1 14. The *Lactobacillus* bacterium of claim 1, wherein the biologically-
2 active polypeptide is between 10 and 600 amino acids.
- 1 15. The *Lactobacillus* bacterium of claim 1, wherein the biologically
2 active protein binds to a pathogen when the biologically active protein is contacted with the
3 pathogen.
- 1 16. The *Lactobacillus* bacterium of claim 15, wherein the pathogen is a
2 bacterial pathogen.
- 1 17. The *Lactobacillus* bacterium of claim 15, wherein the pathogen is a
2 fungal pathogen.
- 1 18. The *Lactobacillus* bacterium of claim 15, wherein the pathogen is a
2 viral pathogen.
- 1 19. The *Lactobacillus* bacterium of claim 18, wherein the viral pathogen is
2 HIV.
- 1 20. The *Lactobacillus* bacterium of claim 19, wherein the biologically
2 active protein is CD4 or an HIV-binding fragment of CD4.
- 1 21. The *Lactobacillus* bacterium of claim 19, wherein the biologically
2 active protein is 2D-CD4.

1 22. The *Lactobacillus* bacterium of claim 18, wherein the biologically
2 active protein is cyanovirin-N or a virus-binding fragment of cyanovirin-N.

1 23. The *Lactobacillus* bacterium of claim 18, wherein the viral pathogen is
2 herpes simplex virus.

1 24. The *Lactobacillus* bacterium of claim 18, wherein the biologically
2 active protein is herpes simplex virus entry mediator C (HveC) or a virus-binding fragment of
3 HveC.

1 25. The *Lactobacillus* bacterium of claim 1, wherein the biologically
2 active polypeptide is released from the *Lactobacillus* bacterium.

1 26. The *Lactobacillus* bacterium of claim 4, wherein the biologically
2 active polypeptide is anchored to the cell wall of the *Lactobacillus* bacterium.

1 27. A method of expressing a biologically active polypeptide in the cell
2 wall of a *Lactobacillus* bacterium, the method comprising
3 providing a *Lactobacillus* bacterium comprising an expression cassette, the
4 expression cassette comprising a promoter operably linked to a polynucleotide encoding a
5 signal sequence and a biologically-active polypeptide, wherein the biologically active
6 polypeptide is linked to a heterologous carboxyl terminal cell wall targeting region and
7 wherein the heterologous carboxyl terminal cell wall targeting region comprises in the
8 following order:

9 a cell wall associated sequence;
10 LPQ(S/A/T)(G/A);and
11 a hydrophobic sequence; and
12 culturing the bacterium under conditions to induce expression of the
13 polypeptide, thereby expressing a biologically active polypeptide in the cell wall of a
14 *Lactobacillus* bacterium.

1 28. The method of claim 27, wherein the cell wall associated sequence
2 comprises at least 50 amino acids.

1 29. The method of claim 27, wherein the cell wall associated sequence
2 comprises at least 200 amino acids.

- 1 30. The method of claim 27, wherein the heterologous carboxyl terminal
2 cell wall targeting region further comprises a charged sequence at the carboxyl terminus of
3 region.
- 1 31. The method of claim 27, wherein the providing step comprises
2 transferring the expression cassette into the bacterium.
- 1 32. The method of claim 27, wherein the cell wall targeting region
2 comprises the amino acid sequence LPQSG.
- 1 33. The method of claim 27, wherein the cell wall targeting region
2 comprises the amino acid sequence LPQAG.
- 1 34. The method of claim 27, wherein the cell wall targeting region
2 comprises the amino acid sequence LPQTG.
- 1 35. The method of claim 27, wherein the cell wall targeting region
2 comprises the amino acid sequence LPQTA.
- 1 36. The method of claim 27, wherein the cell wall targeting region
2 comprises SEQ ID NO:7.
- 1 37. The method of claim 27, wherein the cell wall targeting region
2 comprises SEQ ID NO:8.
- 1 38. The method of claim 27, wherein the cell wall targeting region
2 comprises at least 200 amino acids.
- 1 39. The method of claim 27, wherein the *Lactobacillus* bacterium is a
2 vagina-colonizing strain.
- 1 40. The method of claim 27, wherein the *Lactobacillus* bacterium is
2 selected from the group consisting of *L. jensenii*, *L. gasseri*, and *L. casei*.
- 1 41. The method of claim 27, wherein the biologically-active polypeptide is
2 between 10 and 600 amino acids.

- 1 42. The method of claim 27, wherein the biologically active protein binds
2 to a pathogen when the biologically active protein is contacted with the pathogen.
- 1 43. The method of claim 42, wherein the pathogen is a bacterial pathogen.
- 1 44. The method of claim 42, wherein the pathogen is a fungal pathogen.
- 1 45. The method of claim 42, wherein the pathogen is a viral pathogen.
- 1 46. The method of claim 45, wherein the viral pathogen is HIV.
- 1 47. The method of claim 46, wherein the biologically active protein is CD4
2 or an HIV-binding fragment of CD4.
- 1 48. The method of claim 46, wherein the biologically active protein is 2D-
2 CD4.
- 1 49. The method of claim 45, wherein the biologically active protein is
2 cyanovirin-N or a virus-binding fragment of cyanovirin-N.
- 1 50. The method of claim 45, wherein the viral pathogen is herpes simplex
2 virus.
- 1 51. The method of claim 45, wherein the biologically active protein is
2 herpes simplex virus entry mediator C (HveC) or a virus-binding fragment of HveC.
- 1 52. The method of claim 27, wherein the biologically active polypeptide is
2 released from the *Lactobacillus* bacterium.
- 1 53. The method of claim 30, wherein the biologically active polypeptide is
2 anchored in the cell wall of the *Lactobacillus* bacterium.
- 1 54. A method of providing a biologically active protein to a mammalian
2 mucosal surface, the method comprising,
3 contacting a mucosal surface with a *Lactobacillus* bacterium recombinantly
4 altered to express a signal sequence linked to a biologically-active polypeptide linked to a
5 heterologous carboxyl terminal cell wall targeting region, the heterologous carboxyl terminal
6 cell wall targeting region comprising in the following order:

7 a cell wall associated sequence;
8 LPQ(S/A/T)(G/A); and
9 a hydrophobic sequence,
10 wherein the biologically active polypeptide is expressed in an amount able to
11 be detected in a sample collected from the mucosal surface.

1 55. The method of claim 54, wherein the cell wall associated sequence
2 comprises at least 50 amino acids.

1 56. The method of claim 54, wherein the cell wall associated sequence
2 comprises at least 200 amino acids.

1 57. The method of claim 54, wherein the heterologous carboxyl terminal
2 cell wall targeting region further comprises a charged sequence at the carboxyl terminus of
3 region.

1 58. The method of claim 54, wherein the *Lactobacillus* bacterium is
2 selected from the group consisting of *L. jensenii*, *L. gasseri*, and *L. casei*.

1 59. The method of claim 54, wherein the mucosal surface resides within
2 the vagina.

1 60. The method of claim 54, wherein the mucosal surface resides within
2 the gastrointestinal tract.

1 61. The method of claim 54, wherein the contacting step comprises orally
2 administering the *Lactobacillus* bacterium.

1 62. The method of claim 54, wherein the contacting step comprises
2 vaginally administering the *Lactobacillus* bacterium.

1 63. The method of claim 54, wherein the contacting step comprises
2 rectally administering the *Lactobacillus* bacterium.

1 64. An expression cassette comprising a promoter operably linked to a
2 polynucleotide encoding a signal sequence and a biologically-active polypeptide, wherein the
3 biologically active polypeptide is linked to SEQ ID NO:7 or SEQ ID NO:8.

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65. A vector comprising the expression cassette of claim 64.